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Arsenic metabolism in a freshwater food chain: blue–green alga (*Nostoc* sp.)→ shrimp (*Neocaridina denticulata*)→ carp (*Cyprinus carpio*)

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Bioaccumulation and biomethylation of inorganic arsenic were investigated in a three-step freshwater food chain consisting of an autotroph (blue–green alga: *Nostoc* sp.), a herbivore (shrimp: *Neocaridina denticulata*) and a carnivore (carp: *Cyprinus carpio*). The autotroph, herbivore and carnivore survived in arsenic-containing water below 1000, 2 and 60 mg As(V) dm^{-3}, respectively. Bioaccumulation of arsenate by *Nostoc* sp. was decreased with an increase in the nitrogen concentration of the medium. Arsenic(V) was accumulated from the water phase and part-methylated by the carp, as well as by the algae and shrimp. Arsenic was mostly accumulated in the gut of the carp. The predominant arsenical in the guts was the monomethylarsenic species.

Arsenic accumulation via food in the above three-step food chain decreased by one order of magnitude and the relative concentration of methylated arsenic to the total arsenic accumulated increased successively with an elevation in the trophic level. When arsenicals were transferred via the food chain, no monomethylarsenic, or only a trace amount, was detected in the three organisms. Dimethylarsenic in the alga, both dimethyl- and trimethyl-arsenic in shrimp, and trimethylarsenic in carp, were the predominant methylated arsenic species, respectively.

Keywords: Arsenic, accumulation, methylation, freshwater organisms, alga, shrimp, carp, food chain

INTRODUCTION

Many papers on biotransformation of arsenic via marine food chains have been published. For example, three trophic levels of marine organisms (phytoplankton—*Dunaliella marina*, zooplankton—*Artemia salina*, and shrimp—*Lysmata seticaudata*) were tested for their arsenic metabolism.[1] The experimental results led to the conclusion that organic forms of arsenic in marine food webs were derived from an *in vivo* synthesis by primary producers and were efficiently transferred along a marine food chain. The shrimp, the highest trophic level in this food chain, could not form organic arsenic by itself. In this case, arsenate taken up from water was converted largely to arsenite. Similar conclusions were reached from the experimental results on a phytoplankton–mussel (*Mytilus galloprovincialis*)–crab (*Carcinus marnas*) system,[2] a phytoplankton (*Fucus spiralis*)–grazer snail (*Littorina littoralis*)–carnivole snail (*Nucella laillus*) system,[3,4] and a phytoplankton (*Dunaliella tertiolecta*)–lobster (*Homarus americanus* juveniles) system.[5] More information about biotransformation of arsenic in marine ecosystems is available in a review.[6]

There have been very few papers on biotransformation of arsenic in freshwater ecosystems. Isensee *et al.*[7] examined the distribution of ^{14}C-labeled cacodylic acid (CA) and dimethylarsine (DMA) among freshwater organisms in a model ecosystem. Fish, *Daphnia magna*, snails and algae were exposed to CA and DMA for 3, 29, 32 and 32 days, respectively. The freshwater organisms represented parts of two food chains: water→algae→snails; and water→diatoms, protozoa, and rotifers→*Daphnia*→ fish. The results showed that lower food chain organisms (algae and *Daphnia*) bioaccumulated more CA and DMA, and amounts accumulated indicate that CA and DMA do not show a high potential to biomagnify in the environment.

Lindsay and Sanders[8] reported arsenic uptake and transfer in a simplified estuary river water (salinity 11.5–15‰) containing 0.2–1.0 μg As dm^{-3}, which was enriched with arsenate at con-

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centrations of 10 and 25 μg As(V) dm^{-3}. Three phytoplanktons (*Thalassiosira pseudonana*, *Skeletonema costatum* and *Dunaliella tertiolecta*) were cultured in the arsenate-enriched river water (25 μg As(V) dm^{-3}). Brine shrimp (grazer, *Artemia* sp.) was exposed to waterborne arsenic or to arsenic-loaded phytoplankton, and grass shrimp (carnivore, *Palaemonetes pugio*) was exposed to waterborne arsenic or to arsenic-dosed *Artemia*. They concluded that uptake of arsenic by phytoplankton was significant; however, only small amounts of arsenic were incorporated by *Artemia* and no significant increases in arsenic content of *P. pugio* were found in those organisms exposed to arsenic-containing food or to arsenic-enriched waters.

Saito and Takahashi[9] analyzed arsenic in sweetfish living in a river polluted with arsenic from hot springs (Takinoue Hot Spring, Iwate Prefecture, Japan). Arsenic concentrations were 14.3 μg As g^{-1} in sediment, 261 μg As g^{-1} in moss, and 2.24 μg As g^{-1} in river water. However, arsenic was not detected in the algae-eating sweetfish.

We have previously reported the transformation of inorganic arsenic compounds in freshwater food chains starting from autotrophs (microalgae: *Chlorella vulgaris*[10–12] and *Phorimidium* sp.[11]) through grazers (zooplankton: *Moina macrocopa*[10–12] and herbivorous shrimp: *Neocaridina denticulata*[12]) to carnivores (goldfish: *Carassius carassius auratus*[10] and guppy: *Poecilia reticulata*[11,12]). These experimental results showed that the total arsenic concentration decreased by one order of magnitude whilst the relative concentration of methylated arsenic to the total arsenic, on the contrary, increased successively with an elevation in the trophic level.

The results obtained from marine organisms and those from freshwater organisms resemble each other, in that herbivorous and carnivorous organisms accumulate predominantly dimethylarsenic and trimethylarsenic in their food chains, respectively. The experimental results from freshwater food chains show that the total arsenic concentration in organisms decreases with an elevation in the trophic level; this situation is the same as the results from marine food chains.[6]

This paper presents the arsenic accumulations from the water phase by a blue–green alga (*Nostoc* sp.), shrimp (*Neocaridina denticulata*) and carp (*Cyprinus carpio*), and via a new three-step freshwater food chain starting from an autotroph, *Nostoc* sp., through a herbivorous shrimp, *Neocaridina denitculata*, to carnivorous *Cyprinus carpio*. This three-step food chain is closer to that in the natural environment than those previously reported.[10–12]

EXPERIMENTAL

Culture of organisms

The organisms tested were cultured or fed under the following conditions.

Autotrophic blue–green alga

A suspension (6 mg dry algal mass in 5 cm^3 suspension per dm^3 medium) of *Nostoc* sp. which was isolated from an arsenic-polluted environment[13] was placed in an MA medium [$Ca(NO_3)_2 \cdot 4H_2O$ 50, KNO_3 100, $NaNO_3$ 50, Na_2EDTA 5, bicine 500, disodium β-glycerophosphate 100, $CoCl_2 \cdot 6H_2O$ 5, $FeCl_3 \cdot 6H_2O$ 0.5, $Na_2MoO_4 \cdot 2H_2O$ 0.8, $MgCl_2 \cdot 6H_2O$ 50, $MnCl_2 \cdot 4H_2O$ 5, $ZnCl_2$ 0.5, H_3BO_3 20, Na_2SO_4 40 mg dm^{-3}] containing a set amount of arsenic [as elemental arsenic for $Na_2HAsO_4 \cdot 7H_2O$, abbreviated as As(V)]. The alga was cultured at room temperature (14–30 °C) under constant aeration (2 dm^3 min^{-1}) and illumination (4000 lux, 12 h day^{-1}) for a set number of days.

The cells were harvested, and washed by mixing them with distilled–deionized water and separated by centrifugation; this procedure was repeated twice more. The washed wet cells were dried at 60 °C to constant weight.

Herbivorous shrimp

A shrimp (*Neocaridina denticulata*: 1.5 cm in length) was collected from a natural stream in Kagoshima prefecture.[12] The shrimp was fed with a basic diet (Tetrafin, manufactured in Germany) in aerated diluted MA medium (medium/distilled water, 1:25 v/v). The shrimp was harvested by plankton net, washed with the pure water and dried at 60 °C to constant weight.

In the food chain experiment, the shrimp was fed with dried powder of the arsenic-dosed *nostoc* sp.

Carnivorous carp

Carp (*Cyprinus carpio* Linné: juvenile, about 10 cm in length) were obtained from Kagoshima Prefectural Fishery Experiment Station. Two carp were each fed for seven-day periods with Tetrafin in 5 dm^3 of, eight diluted (1:25) MA

media containing 0, 10, 20, 30, 40, 50, 60 and 70 μg As(V) cm^{-3}. One of the two carp did not survive in a medium containing the maximum concentration of 70 μg As(V) cm^{-3}. The carp were harvested, washed with pure water, and cut into three parts: meat; gut; and the rest, including skin, scale, bone and fin. Each part was dried at 60 °C to constant weight, ground into powder and analyzed for total and methylated arsenic. The dry mass ratio of meat/guts/remnants was about 4:1:6.

In the food chain experiment, *C. carpio* was fed with the dried powder of *N. denticulata* that had accumulated arsenic via arsenic-dosed *Nostoc* sp.

Determination of total and methylated arsenic compounds

For the determination of total arsenic in organisms, the dried organisms (10–20 mg) were mineralized in the presence of magnesium nitrate, the ash was dissolved with 10 mol dm^{-3} hydrochloric acid (10 cm^3) with 40% potassium iodide (1 cm^3), the solution was extracted with chloroform (5 cm^3), the chloroform phase was back-extracted with 0.02% magnesium nitrate aqueous solution (2 cm^3) and the aqueous phase was analyzed by graphite-furnace atomic absorption spectrometry. Disodium arsenate [$Na_2HAsO_4 \cdot 7H_2O$: As(V)] was used as an authentic sample for total and nonmethylated arsenic compounds.

For the determination of methylated arsenic compounds, the dried organism (*ca* 10 mg) was digested with 2 mol dm^{-3} NaOH (5 cm^3) at 90–95 °C for 3 h using an aluminum heating block (hot-base digestion). Methylated arsenic compounds in the digest were reduced with $NaBH_4$ to the arsine compounds. The arsines generated were at once frozen out in a liquid-nitrogen U-trap. When the U-trap was wormed, the arsines borne out of it successively were passed through a quartz-tube atomizer and determined chromatographically using an atomic absorption spectrometer on the basis of the difference in the boiling points of the arsines.

Methylarsonic acid, dimethylarsinic acid and arsenobetaine were used as authentic samples for monomethyl-, dimethyl- and trimethyl-arsenic compounds (abbreviated as MMA, DMA and TMA), respectively. These three methylated compounds were degraded to monomethyl-, dimethyl- and trimethyl-arsine oxides upon hot-base digestion, and then hydrided to monomethyl-, dimethyl- and trimethyl-arsines on treatment with sodium borohydride, respectively. Nonmethylated arsenic (abbreviated as IA) concentration was calculated as total arsenic minus the sum of methylated arsenic (MMA + DMA + TMA). The concentrations of all arsenic compounds are expressed as μg As g^{-1}.

Detection limits for total and methylated arsenic, and error limits, were 5 ng and 5%, respectively.

RESULTS AND DISCUSSION

Growth curve and arsenic accumulation by *Nostoc* sp.

A *Nostoc* sp. suspension (25 cm^3, 30 mg dry mass) was placed in an MA medium (5 dm^3) containing 100 mg As(V) and cultured at room temperature (22 ± 2 °C in November) for 24 days. The algal sample was drawn from the culture at set intervals and analyzed for growth and arsenic accumulation. The experimental results are shown in Fig. 1. The solid curve in Fig. 1 is calculated by the following logistic equation:

$$y = \frac{CM}{1 + (M-1)\exp[-K(x-W)]} \quad [1]$$

where y is algal growth (g dry wt dm^{-3} medium), x is culture time (days), C is initial algal concentration (about 0.006 g dm^{-3}, on a dry basis), M is the multiplication factor of the cell (the cell ratio of final algal concentration to initial algal concentration), and K and W are the growth parameters, which were chosen, so as to minimize the devi-

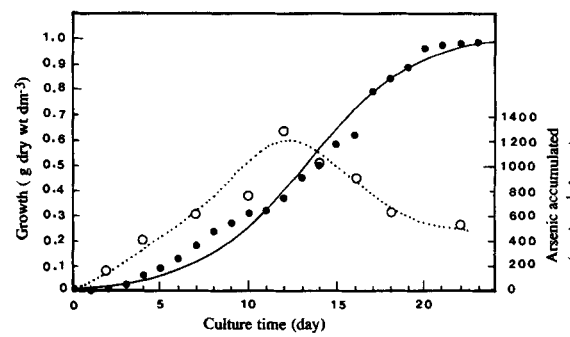

Figure 1 Growth curve and arsenic bioaccumulation of blue-green alga, *Nostoc* sp., cultured at 22 ± 2 °C in 5 dm^3 medium: ●, growth observed; solid curve, theoretical logistic curve (Eqn [1]); ○, arsenic accumulated; curve fitted to experimental points.

ation between the observed data and the calculated curve from Eqn [1] above, to be 0.335 and −2, respectively. Figure 1 shows that the algal growth data were approximated well by the logistic curve in the same manner as the growth of algae *Chlorella vulgaris*[12,14] and *Phormidium* sp.[15] in the medium containing As(V), and *C. vulgaris* in the medium containing As(III).[16]

Arsenic(V) accumulation by *Nostoc* sp. increased with an increase in the culture time, reached a peak at the exponential growth phase and then decreased in like manner in the case of *C. vulgaris*, as described in the previous paper.[12,14] The decrease of arsenic accumulation indicates that the rate of arsenic excretion exceeded the rate of arsenic take-up after the exponential growth phase.

Effect of arsenic(V) concentration in medium on arsenic accumulation and biomethylation by *Nostoc* sp.

A *Nostoc* cell suspension (5 cm^3, 6 mg dry wt) was placed in MA medium (1 dm^3) containing different levels of As(V) and cultured at room temperature (28 ± 2 °C in July) for two weeks under general conditions. The cells harvested were analyzed for growth, total arsenic and methylated arsenic species. The experimental results are shown in Table 1.

Table 1 shows that gowth of *Nostoc* sp. at the exponential growth phase (two weeks' culture) increased with an increase in arsenic(V) concentrations in media of up to 100 μg As(V) cm^{-3} and then decreased in the media at arsenic(V) concentrations higher than 200 μg cm^{-3}. In the previous paper,[9] maximum *Nostoc* cell growth (1.2 g dry wt dm^{-3}) at the stationary growth phase (32 days' culture) was observed in MA medium at an arsenic(V) concentration of 1 μg cm^{-3} in the range 0–1000 μg cm^{-3}. The authors suppose that the difference in arsenic concentration in medium giving the maximum algal growth was caused by the difference in growth phases of both the algae analyzed.

From Table 1, arsenate seems to have stimulating effect for the growth of *Nostoc* sp. in MA medium at arsenic concentrations below 100 μg As(V) cm^{-3}.

Arsenic accumulation increased with an increase in arsenic(V) concentration in medium containing up to 500 μg As(V) cm^{-3}. A very small fraction of the accumulated arsenic was biomethylated to dimethylarsenic compounds. The amount of dimethylarsenic in the cells tended to increase with an increase in the arsenic concentration of the medium, but the relative concentration remained constant at 0.3–0.9%. Monomethylarsenic was detected but in trace amounts, and no trimethylarsenic was detected in the cells. *Nostoc* sp. was found to have a mechanism for biomethylating arsenate but the ability was limited, as shown in other algae.[10–12,15]

Effect of nitrogen nutrients on growth, arsenic accumulation and biomethylation by *Nostoc* sp.

Nostoc sp. cells were inoculated into MA medium (0.5 dm^3) containing variable levels of nitrogen nutrients (0–140 μg N cm^{-3}) and a constant level of As(V) (100 μg cm^{-3}), and cultured at room

Table 1 Effect of arsenic(V) concentration in MA medium on arsenic accumulation and biomethylation by *Nostoc* sp. cultured at 28 ± 2 °C for two weeks in 1 dm^3 medium

As(V) in medium (μg cm^{-3})	Growth (g dry wt dm^{-3})	Arsenic in *Nostoc* sp. [μg As g^{-1} dry wt (%)]				
		Total	IA	MMA	DMA	TMA
1	0.64	18	18 (100)	tr	tr	—
10	0.76	163	162 (99.7)	tr	0.7 (0.3)	—
50	0.74	572	565 (99.5)	tr	3.0 (0.5)	—
100	0.88	785	778 (99.1)	tr	7.1 (0.9)	—
200	0.56	1380	1370 (99.1)	tr	12.7 (0.9)	—
500	0.40	1569	1560 (99.4)	tr	9.8 (0.6)	—

Abbreviations: IA, nonmethylated arsenic; MMA, monomethylarsenic; DMA, dimethylarsenic; TMA, trimethylarsenic; tr, detected but trace amount; —, not detected.
IA(%) = Total(%) − (MMA + DMA + TMA)(%).

Table 2 Effect of nitrogen concentration in MA medium on growth, arsenic accumulation and biomethylation in *Nostoc* sp. cultured at 16 ± 2 °C for two weeks in 0.5 dm^3 medium

Nitrogen in medium ($\mu g\ cm^{-3}$)	Growth (g dry wt dm^{-3})	Arsenic in *Nostoc* sp. [$\mu g\ As\ g^{-1}$ dry wt (%)]				
		Total	IA	MMA	DMA	TMA
0	0.49	840	820 (97.5)	4.2 (0.5)	16.4 (2.0)	tr
2.8	0.56	588	580 (99.1)	tr	5.3 (0.9)	tr
28[a]	0.63	543	540 (99.2)	tr	4.2 (0.8)	—
56	0.68	482	480 (99.4)	tr	3.0 (0.6)	—
140	0.39	179	180 (100)	tr	tr	—

Abbreviations: as shown in Table 1.
[a] The regular nitrogen concentration in MA medium.

Table 3 Accumulation and methylation of inorganic arsenic (V) by shrimp, *N. denticulata*, from arsenic-containing water[a]

As(V) in water ($mg\ As\ dm^{-3}$)	Concentration of arsenic in shrimp [$\mu g\ As\ g^{-1}$ dry wt (%)]				
	Total	IA	MMA	DMA	TMA
0.1	18.9 (100)	15.9 (84.1)	—	1.9 (10.1)	1.1 (5.8)
0.2	18.5 (100)	14.9 (80.5)	tr	1.9 (10.3)	1.7 (9.2)
0.3	19.8 (100)	17.3 (87.4)	—	1.4 (7.1)	1.1 (5.5)
0.5	22.6 (100)	15.4 (68.1)	tr	2.6 (11.5)	4.6 (20.4)
1.0	33.2 (100)	30.2 (91.0)	tr	1.7 (5.1)	1.3 (3.9)
1.5	31.6 (100)	27.9 (88.3)	tr	2.2 (7.0)	1.5 (4.7)

Abbreviations: as shown in Table 1.
[a] Data are reproduced from our previous paper.[12]

Table 4 Accumulation and methylation of inorganic arsenic(V) by carp, *C. carpio* (meat), from arsenic-containing water

Arsenic (V) in medium ($\mu\ cm^{-3}$)	Arsenic in *C. carpio* [$\mu g\ As\ g^{-1}$ dry wt (%)]				
	Total	IA	MMA	DMA	TMA
0	2.0	1.8 (90.0)	—	—	0.2 (10.0)
10	3.8	3.6 (94.7)	tr	tr	0.2 (5.3)
20	6.0	5.0 (83.3)	0.4 (6.7)	0.2 (3.3)	0.4 (6.7)
30	5.8	4.6 (79.4)	0.2 (3.4)	0.1 (1.7)	0.9 (15.5)
40	7.2	6.0 (83.3)	0.5 (6.9)	0.3 (4.2)	0.4 (5.6)
50	11.4	7.0 (61.4)	3.1 (27.2)	0.6 (5.3)	0.7 (6.1)
60	12.0	7.1 (59.2)	2.5 (20.8)	1.0 (8.3)	1.4 (11.7)
70[a]	11.6	9.8 (84.5)	0.5 (4.3)	0.4 (3.4)	0.9 (7.8)

Abbreviations: as shown in Table 1.
[a] One of two carp did not survive.

temperature (16 ± 2 °C in December) for two weeks under general conditions. Growth, arsenic accumulation and biomethylation in the cells were determined.

The results are summarized in Table 2: growth increased with an increase in nitrogen concentration in the medium up to 56 $\mu g\ N\ cm^{-3}$; regular MA medium contains $Ca(NO_3)_2 \cdot 4H_2O$ 50, KNO_3 100 and $NaNO_3$ 50 mg dm^{-3} as nitrogen nutrients, amounting to 28 $\mu g\ N\ cm^{-3}$. On the other hand, the arsenic concentrations of all the chemical species in the cells decreased with an increase in

Table 5 Accumulation and methylation of inorganic arsenic(V) by carp, *C. carpio* (gut), from arsenic-containing water

Arsenic (V) in medium ($\mu g\ cm^{-3}$)	Arsenic in *C. carpio* [$\mu g\ As\ g^{-1}$ dry wt (%)]				
	Total	IA	MMA	DMA	TMA
0	7.6	7.3 (96.1)	—	0.2 (2.6)	0.1 (1.3)
10	19.7	15.0 (76.2)	3.8 (19.3)	0.6 (3.0)	0.3 (1.5)
20	23.8	16.0 (60.0)	4.8 (20.2)	1.4 (5.9)	1.9 (7.9)
30	40.0	13.0 (32.5)	24.0 (60.0)	1.4 (3.5)	1.6 (4.0)
40	51.4	17.0 (33.1)	29.0 (56.4)	3.4 (6.6)	2.0 (3.9)
50	60.6	20.0 (33.2)	36.0 (59.4)	3.1 (5.1)	1.5 (2.5)
60	82.8	22.0 (26.6)	57.0 (68.8)	1.5 (1.8)	2.3 (2.8)
70[a]	60.4	44.0 (72.9)	13.0 (21.5)	1.8 (3.0)	1.6 (2.6)

Abbrevitions: as shown in Table 1.
[a] One of two carp did not survive.

Table 6 Accumulation and methylation of inorganic arsenic(V) by carp, *C. carpio* (remnants including skin, scale, bone and fin), from arsenic-containing water

Arsenic in medium ($\mu g\ cm^{-3}$)	Arsenic in *C. carpio* [$\mu g\ As\ g^{-1}$ dry wt (%)]				
	Total	IA	MMA	DMA	TMA
0	5.5	5.4 (98.2)	—	0.1 (1.8)	tr
10	7.5	6.5 (86.7)	0.3 (4.0)	0.1 (1.3)	0.6 (8.0)
20	7.9	6.7 (84.8)	0.4 (5.1)	0.2 (2.5)	0.6 (7.6)
30	6.7	5.2 (77.6)	0.5 (7.5)	0.2 (3.0)	0.8 (11.9)
40	6.8	5.0 (73.5)	0.5 (7.4)	0.4 (5.9)	0.9 (13.2)
50	13.8	9.2 (66.6)	2.7 (19.6)	0.7 (5.1)	1.2 (8.7)
60	12.6	9.0 (71.4)	1.8 (14.3)	0.5 (4.0)	1.3 (10.3)
70*	12.2	9.3 (76.2)	0.8 (6.6)	0.9 (7.4)	1.2 (9.8)

Abbreviations: as shown in Table 1.
[a] One of two carp did not survive.

the nitrogen concentration of the MA medium. The fourth row of data (100 $\mu g\ As\ cm^{-3}$) in Table 1, the third row of data (28 $\mu g\ N\ cm^{-3}$) in Table 2, and the 14th culture data-point in Fig. 1 were obtained experimentally by use of the same nutrient medium but under different culture conditions: the algae were cultured at $28 \pm 2\,^{\circ}C$ in 1 dm^3 medium, at $16 \pm 2\,^{\circ}C$ in 0.5 dm^3 medium, and at $22 \pm 2\,^{\circ}C$ in 5 dm^3 medium, respectively. The variations of the data were caused by the different culture conditions. The growth rate was greater at higher temperatures and in smaller containers.

In our previous paper, *Nostoc* sp. was incubated in buffer solution at pH 7 containing 2 $\mu g\ As(V)\ cm^{-3}$ (constant) and KNO_3 (varying from 0 to 5000 $\mu g\ N\ cm^{-3}$). The previous experiment showed similar results, i.e. the maximum arsenic accumulation was observed in the buffer containing no nitrogen.[13] *Nostoc* sp. possesses a nitrogenase in its heterocyst; this nitrogenase has been reported to be synthesized and activated only in the absence of nitrogen compounds in the medium.

The present and previous results suggested that arsenic accumulation and biomethylation were stimulated by the biosynthesis and activation of the nitrogenase.

Accumulation of arsenic(V) from the water phase by shrimp (*Neocaridina denticulata*)

The data on the accumulation of arsenic(V) from the water phase and the transformation by *N. denticulata* were obtained from our previous experiments[12] and they are summarized in Table 3. The shrimp did not survive in a medium with

2.0 mg As(V) dm^{-3}. Table 3 shows that accumulation of arsenic by *N. denticulata* from the water increased with an increase of the arsenic concentration in the medium up to 1.0 μg As(V) cm^{-3}. The total concentration of arsenic accumulated by *N. denticulata* from the water was comparable with that by *Moina* sp.,[10] but lower than that by goldfish.[10] About 10–30% of the total arsenic accumulated was methylated by *N. denticulata*. The relative concentration of methylated arsenic in the shrimp was much higher than those obtained from algal experiments, including experiments reported in this paper, and comparable with those from *Moina* sp.[10] The predominant methylated arsenicals were dimethyl- and trimethyl-arsenic compounds.

Accumulation of arsenic(V) from the water phase by carp (*Cyprinus carpio*)

The carp, *C. carpio*, containing arsenic accumulated from the water phase was cut into three parts: meat; gut; and the resonants, including skin, scale, bone and fin. Experimental results for the three parts, and the average in the whole body of *C. carpio*, are shown in Tables 4–6, and Fig. 2, respectively.

Arsenic was mostly accumulated in the gut and the arsenic concentration in meat was comparable with that in the resonants. Arsenic accumulation in any part of the body increased with an increase in arsenic concentration in the aqueous phase, except for 70 μg As(V) cm^{-3} (Tables 4–6, and Fig. 2). The methylated arsenic compounds existed in the gut at the highest concentrations. About 70% of the total arsenic accumulated in the gut was biomethylated when carp were exposed to 30 to 60 μg As(V) cm^{-3}. The predominant methylated arsenic species was MMA, an average for whole body (Fig. 2). These experimental data show that *C. carpio* can also take up As(V) directly from the water phase, biomethylate the arsenic and accumulate it mainly in the gut in the form of monomethyl compounds.

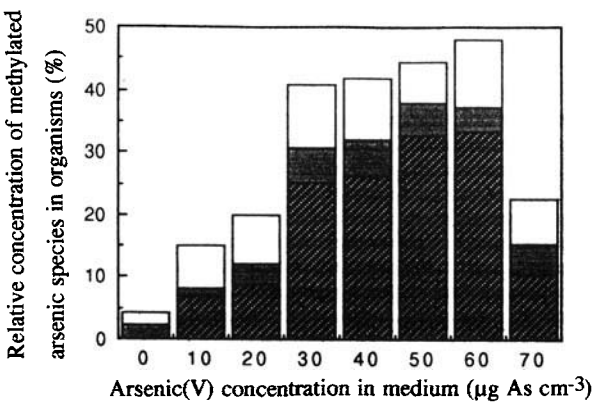

Figure 2 Relative concentration of methylated arsenic to total arsenic compounds accumulated by carp (*C. carpio*: in whole body) via the water phase. Arsenic concentrations in the whole body of the carp were calculated from arsenic concentrations (%) in each of the three parts shown in Tables 4–6 and the weight ratios of these parts. □ MMA, □ DMA, □ TMA.

Biotransformation of arsenic(V) in a food chain of alga (*Nostoc* sp.)→shrimp (*N. denticulta*)→carp (*C. carpio*)

Alga *Nostoc* sp. was cultured in MA medium containing 100 μg As(V) cm^{-3} for two weeks; the arsenic-dosed algal cells were harvested and washed with distilled water. Ten shrimps (*N. denticulata*: 1.5 cm long, 15 mg dry wt each) were fed for seven days with the dry powder of the arsenic-dosed alga (about 5 mg dry wt per 10 shrimps per day: 35 mg total) in aerated, diluted MA medium (1 dm^3: 40 cm^3 MA and 960 cm^3 distilled water), collected and washed with distilled water. Two carp (*C. carpio*: 10 cm long, 4 g dry wt each) were fed for seven days with the dry powder of the shrimp accumulating arsenic via arsenic-dosed alga (about 15 mg dry wt shrimp per two carp per day; 105 mg total) in aerated, diluted MA medium, collected and washed with distilled water. These arsenic-accumulating organisms were analyzed for total and methylated arsenic compounds. The experimental results are summarized in Table 7 and relative concentrations of methylated arsenic species in the organisms are illustrated in Fig. 3.

Table 7 shows that total and inorganic arsenic concentrations in the organisms decreased one order of magnitude successively with an elevation in the trophic level. On the other hand, the relative concentrations of methylated arsenic species to total arsenic compounds increased dramatically, as shown in Fig. 3. No monomethylarsenic compound, or only a trace amount, was detected in the three organisms, and dimethyl- and trimethyl-arsenic compounds were the predominant methylated arsenic species in alga and carp, respectively. Very similar results were reported from our previous work on the food chains *C. vulgaris*→moina→goldfish[10] and *C. vulgaris*→moina→guppy.[11,12] These results indicate that the lower trophic level of organisms has

Table 7 Biotransformation of arsenic(V) in a food chain of alga (*Nostoc* sp.)→shrimp (*N. denticulata*)→carp (*C. carpio*)

Organisms[a]	Arsenic in organisms [μg As g^{-1} dry wt (%)]				
	Total	IA	MMA	DMA	TMA
Nostoc sp.	743	736 (99.1)	tr	6.7 (0.9)	—
N. denticulata	11.6	10.4 (89.6)	—	0.6 (5.2)	0.6 (5.2)
C. carpio	5.2	4.1 (78.8)	tr	0.4 (7.7)	0.7 (13.5)

Abbreviations: as shown in Table 1.

[a] *Nostoc* sp. was cultured in MA medium containing 100 mg As(V) dm^{-3} for two weeks, *N. denticulata* was fed for seven days with the dried powder of the arsenic-dosed *Nostoc* sp., and *C. carpio* was fed for seven days with dried powder of the *N. denticulata* which had accumulated arsenic via arsenic-dosed *Nostoc* sp.

a greater ability to accumulate arsenic and the higher trophic level of organisms has a greater ability to methylate arsenic.

It is similarly observed in seawater ecosystems that the arsenic bioaccumulation decreases via food chains.[6] That is, arsenic concentrations are not biomagnified in the aquatic food chain; this is in striking contrast to other heavy metals and toxic substances such as mercury, methylmercury, dichlorodiphenyltrichloroethane (DDT) and so on.

However, the proportion of biomethylated arsenic species in the higher levels of organisms in this freshwater food chain is considerably different from those in plants such as kelp and in most animals in seawater ecosystems. In plants and carnivores in marine ecosystems[16, 17] the greater part (>90%) of accumulated arsenic is methylated, and methylated arsenic species are almost all in the form of dimethyl- and trimethyl-arsenic compounds, respectively.

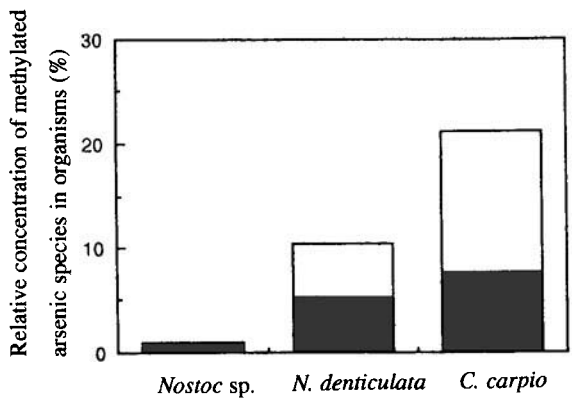

Figure 3 Relative concentration of methylated arsenic to total arsenic compounds accumulated by organisms via the food chain: blue-green alga (*Nostoc* sp.)→shrimp (*N. denticulata*)→carp. (*C. carpio*). ■DMA, □TMA.

Although substantial contributions concerning the distribution and nature of arsenicals in the marine environment had been made by Lunde,[18] it was not until 1977 that arsenobetaine and arseno-sugars were isolated from rock lobster[19] and kelp[20] by Edmonds and Francesconi. Since these discoveries, arsenobetaine and the arseno-sugars have been shown to be present, and to be the most abundant arsenicals, in most marine animals and plants, respectively, so far investigated.

On the basis of the experimental data obtained from marine organisms, Edmonds and Francesconi[21] proposed a biochemical pathway for arsenic methylation. They noted that the structure of arseno-sugars with the ribose configuration was consistent with their formation from *S*-adenosylmethionine (SAM). Here SAM was assumed to switch its role from being a CH_3^+ source to being a donor of the adenosyl group.

Very few papers on the organic chemical structure of arsenic compounds in freshwater organisms have been published. Norin *et al.*[22] reported that arsenobetaine and arsenocholine were detected in shrimps from arsenic-polluted lake water and unpolluted waters. They proposed a possible biochemical pathway from arseno-sugar to arsenobetaine through arsenocholine as an intermediate.

The combination of the above two biochemical pathways from dimethylarsenic acid to arsenobetaine is shown in Fig. 4. We also estimate that the methylated arsenic compounds observed in our work were biotransformed as a means of detoxification from arsenate through the pathway shown in Fig. 4. Methylation of arsenic may be a

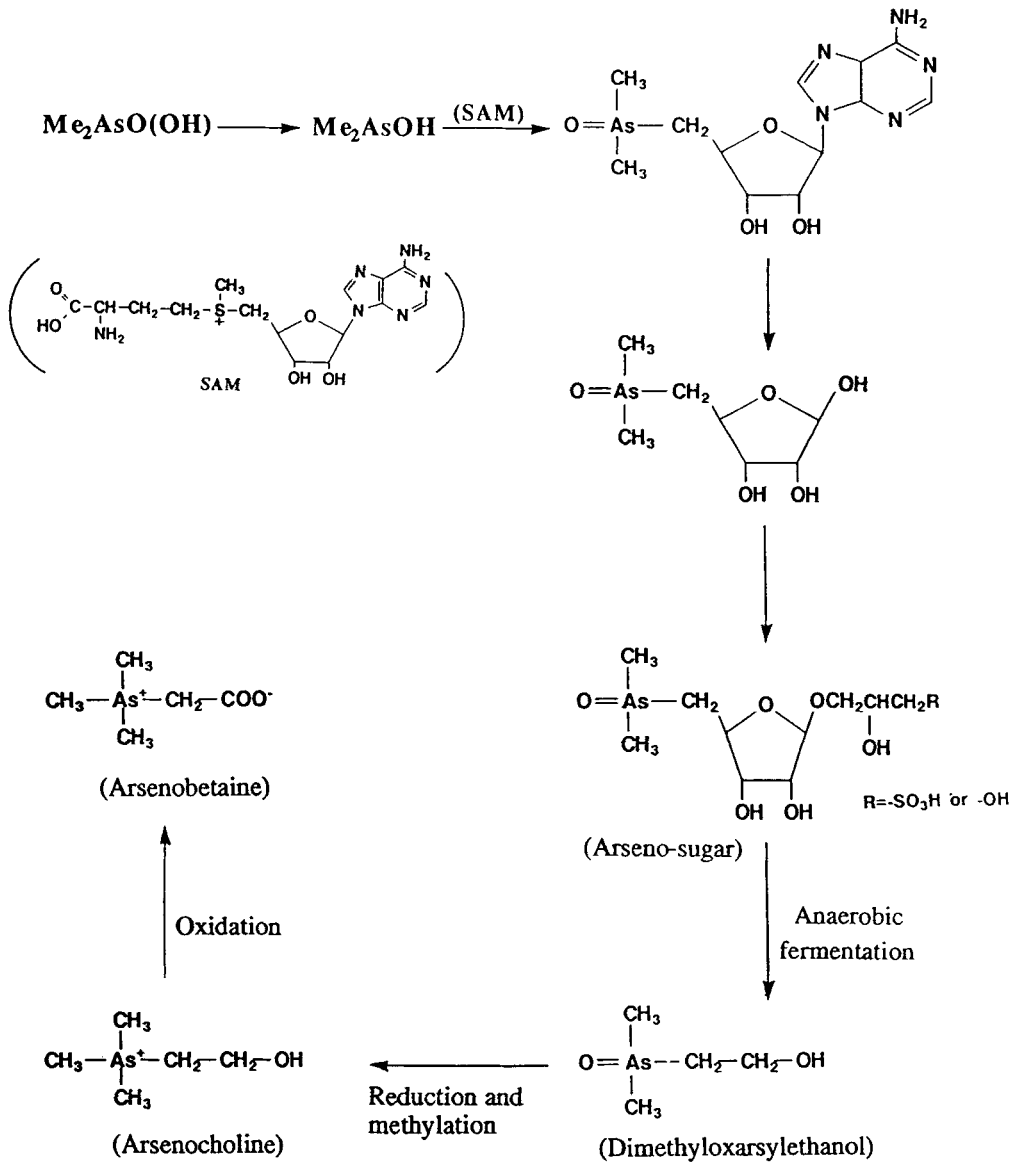

Figure 4 A possible biochemical pathway from dimethylarsinic acid to arseno-sugars and from arseno-sugars to arsenobetaine proposed by Edmonds and Francesconi[21] and Norin *et al.*[22] (modified from Refs 21 and 22).

major detoxification process for marine organisms, and probably for freshwater organisms, too. Freshwater organisms, however, may sustain the other arsenic detoxification mechanisms, because they can retain a considerable proportion of nonmethylated arsenic species accumulated in the cells.

Biomethylation of arsenic by freshwater organisms was proved experimentally as mentioned before. The original chemical structures of the arsenic compounds *in situ* in the cells of our freshwater organisms, however, have not yet been revealed. It is necessary, in order to elucidate the biochemical pathway of arsenic methylation, to reveal the nascent chemical forms of the methylated arsenic compounds in freshwater organisms. This is now under investigation.

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